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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/853,257	05/10/2001	Bonnie L. Bassler	PUNIV.002A	5035

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Diane McKay, Esq.
Mathews, Collins, Shepherd & McKay, P.A.
100 Thanet Circle, Suite 306
Princeton, NJ 08540-3674

EXAMINER	
DUFFY, PATRICIA ANN	
ART UNIT	**PAPER NUMBER**
1645	

DATE MAILED: 06/07/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/853,257	BASSLER ET AL.	
	Examiner	Art Unit	
	Patricia A. Duffy	1645	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *17 March 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-53* is/are pending in the application.

4a) Of the above claim(s) *1-8, 13-53* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *9-12* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *5-02; 10/01*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

The response filed 3-17-04 has been entered into the record.

Priority

Applicant's claim for domestic priority under 35 U.S.C. 119(e) is acknowledged.

Specification

The lengthy specification has not been checked to the extent necessary to determine the presence of all possible minor errors. Applicant's cooperation is requested in correcting any errors of which applicant may become aware in the specification.

Applicant is required to cancel the new matter in the reply to this Office Action.

Information Disclosure Statement

The information disclosure statements filed 10-9-01 and 5-3-02 have been considered with the following exceptions. The foreign references numbered 14, 15, 17, 19, and 21 on the disclosure statement of 10-9-01 have only been considered to the extent of the English Abstract. Should Applicants wish the full text of the foreign documents to be considered, a translation into English should be provided. Complete copies of references 18 and 20 were not provided and as such these references have not been considered because they are not in compliance with 37 CFR 1.98(a)(2). Additionally, the Textbooks cited as references 54, 58, 71, 79, 89, 95 in the disclosure statement of 10-9-01 have not been provided and these references have not been considered because they fail to comply with 37 CFR 1.98(a)(2), which requires a legible copy of each U.S. and foreign patent; each publication or that portion which caused it to be listed; and all other information or that portion which caused it to be listed.

Applicant is advised that the date of any re-submission of any item of information contained in this information disclosure statement or the submission of any missing element(s) will be the date of submission for purposes of determining compliance with the requirements based on the time of filing the statement, including all certification requirements for statements under 37 CFR 1.97(e). See MPEP § 609 ¶ C(1).

Election/Restrictions

Applicant's election with traverse of Group IV, claims 9-12 in the response filed 3-17-04 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

Claims 1-8 and 13-53 are withdrawn from consideration as drawn to non-elected inventions.

Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Claims 9-12 are rejected under 35 U.S.C. 101 because the claimed invention is directed to non-statutory subject matter.

The claimed invention is drawn to a process of nature. The process of contacting σ54 polypeptide with a LuxO polypeptide occurs naturally in the process of quorum sensing of *Vibro harveyi* when the *Vibro harveyi* reach certain cell densities and produce certain autoinducers. Processes of nature are not patentable because they do not reflect the

"hand of man" and neither the σ54 or the LuxO polypeptide are purified. While Applicants have discovered how a process of nature works, that does not make the process *per se* patentable. Further, since this process would occur irrespective of location of *Vibrio harveyi*, the limitations of *in vivo* (in a living body or plant) or *in vitro* (in glass such as a test tube) do not obviate this rejection.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:
The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The following is a quotation of the second paragraph of 35 U.S.C. 112:
The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 9-12 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The claims are drawn to methods of regulating the activity of a σ54 polypeptide comprising contacting with a LuxO polypeptide, wherein the contacting is in vivo, in vitro or the σ54 polypeptide is from *V. harveyi*. The teachings of the specification are limited to demonstration of single σ54 polypeptide having SEQ ID NO:2 from V. harveyi encoded by

the *rpoN* gene locus acts in concert with the phosphorylated-LuxO of V. harveyi to provide for repression of the Lux operon by transcriptional activation of an actual repressor protein "X" and transcriptional activation of siderophore genes and rugose colony formation in V. harveyi. The absence of either of such genes provides for a constitutively active lux operon. The specification is devoid of any showing that LuxO directly binds σ54 to control its activity. The following is of note, σ54 is a subunit of the bacterial RNA holoenzyme. For the purposes of this rejection it is noted that "regulating" encompasses both positive and negative regulation of the activity σ54. It is also noted that "the activity of σ54" is neither defined in the claims nor in the specification and is interpreted to be any activity (i.e. binding to polymerase enzyme, binding to DNA promoter sequences, facilitating transcription). Further, the term contacting is not defined in the specification and is interpreted to represent any means to achieve such.

The claims are not enabled for the following reasons. The specification is devoid of any showing that LuxO directly binds σ54 polypeptide or any variant thereof. σ54 is part of a holoenzyme and directs the enzymatic activity to specific genes by binding to specific DNA promotor sequences in the bacterial genome. The specification specifically teaches that LuxO is inactive and only the phosphorylated from of LuxO is active in promoting transcriptional activation of certain genes, but only when bound to DNA. The art teaches that LuxO is a member of two component regulatory family of proteins that active gene transcription in concert with the alternative sigma factor σ54 of RNA polymerase to provide for transcriptional activation of genes. This regulatory family of proteins bind to DNA enhancer sequences upstream of the promoter to which the σ54 subunit of the RNA polymerase binds. The specification lacks any direct or indirect evidence of direct interaction of LuxO in its non-phosphorylated form with σ54 and how this controls the activity of the σ subunit (i.e. binding to promotor DNA or conversion of the "closed" σ54-holopolymerase to an "open" transcriptionally active polymerase).

Further, the art teaches that oligomerization of similar transcriptional activators and nucleotide binding and hydrolysis are essential for regulating the activity of the RNA polymerase, and the specification does not address these issues. The claims do not reflect the necessary DNA structural binding sequences, the oligomerization of LuxO, the phosphorylation of LuxO or the presence of the σ54 in the holoenzyme for activity. The quorum sensing circuit of V. harveyi is "unlike" all other gram negative quorum sensing organisms using a two component phosphorylation/dephosphorylation cascade (Lilley et al., Molecular Microbiology, 36(4):940-954, 2000). Further, there is no evidence that phosphorylated LuxO has the ability transcriptionally activate the genes described in the specification using any fragment of the described σ54 polypeptide of SEQ ID NO:2, any variant of SEQ ID NO:2 or any other known or described σ54 subunit of bacterial RNA polymerase. The specification fails to teach that σ54 polypeptide can be substituted and achieve the requisite regulation of the bacterial σ54-holoenzyme with LuxO. Mere contacting of σ54 polypeptide with LuxO, does not and can not control the activity of the σ54-holoenzyme because it is not phosphorylated and not bound to DNA. The specification lacks any direct data establishing a direct contact between σ54 and LuxO for regulation of the activity of σ54 in the presence or in the absence of the correct DNA sequence. The art fails to teach the direct interaction of any two-component activator in the absence of particular DNA sequences. The art and the specification lack evidence that LuxO regulates in any manner the activity of binding of σ54-holoenzyme or isolated σ54 itself to the DNA promoter sequence recognized by σ54. The specification and art teach that LuxO is part of a complicated quorum sensing circuit that depends upon a two component phosphorylation-dephosphoryation cascade. The activity of LuxO is dependent on kinases and phosphatases encoded by other proteins as is described in detail in the specification. Mere contacting of LuxO to σ54 does not regulate the activity of σ54, without more. This more, is not in the claims and not specifically described by this specification. In particular, the specification fails to describe the LuxO DNA binding

sequences. The specification fails to describe any isolated component assay that utilizes isolated DNA, isolated LuxO and isolated σ54 for detection of transcriptional activation or regulation of "the activity of σ54". The phosphorylated-LuxO biding site on DNA or σ54 is not disclosed by the specification nor the art. The specification does not disclose that non-phosphorylated-LuxO binds DNA and prevents activation of the σ54-holoenzyme. Therefore, it is unclear how to achieve the claimed regulation of activity of σ54 *in vitro* or in vivo by mere contacting the named two individual components recited in the claims.

In the absence of further guidance from Applicants and in view of the reasons set forth supra, it would require undue experimentation to practice the invention as claimed.

Claims 9-12 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

As to claims 9-12, the recitation of "the activity" in the independent claims is indefinite because it lacks antecedent basis in the claim and it is unclear what activity is being regulated.

The claim limitations drawn to *in vivo* or *in vitro* are confusing for the following reasons. First the definition of *in vivo* is a reference to "within the living body" and body is defined as "the material organized substance of an animal". *V. harveyi* is a free living marine luminous bacterium (Bassler et al , Current Opinion in Microbiology, 2:582-587, 1999; page 583, column 2, first full paragraph, first line). As such it is unclear how contacting is done *in vivo* and wherein lies the difference between *in vivo* and *in vitro* for free living microorganisms such as *V. harveyi*. Applicants should clarify this issue for the record.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless -

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 9-12 are rejected under 35 U.S.C. 102(b) as being clearly anticipated by Bassler et al (Journal of Bacteriology, 179(12):4043-4045, June 1997) or Bassler et al (Molecular Microbiology, 13(2)::273-286, 1994) or Freeman et al (Journal of Bacteriology, 181(3):899-906, Feb 1999) or Freeman et al (Molecular Microbiology, 31(2) :665-667, 1999).

The claims are drawn to methods of regulating the activity of a σ54 polypeptide comprising contacting with a LuxO polypeptide, wherein the contacting is *in vivo*, *in vitro* or the σ54 polypeptide is from *V. harveyi*.

Bassler et al (Journal of Bacteriology, 179(12):4043-4045, June 1997) teach the growing of *V. harveyi* et al reporter strain BB120 at low cell density (see Table 1 and column 2, page 4043) which is the same strain used in the instant application. As such, the process of growing *V. harveyi* et al inherently provides for "contacting a LuxO polypeptide" with a σ54 polypeptide, because both of the polypeptides are inherently present in the *V. harveyi* organism and the contacting of phosphorylated LuxO with σ54-holoenzyme is inherently regulated by cell density as specifically stated in the specificaition (page 28). As such, the step of contacting LuxO with σ54 is inherently performed in growing cells at low density.

Bassler et al (Molecular Microbiology, 13(2)::273-286, 1994) teach that cell density directly affects the light production of *V. harveyi* et al reporter strain BB120 (the same strain of the instant specification) via the expression of the lux operon (see page 273,

column 2, first full paragraph and page 277, Figure 3, first panel, open circles). As such, the process of growing *V. harveyi* et al at low density inherently provides for "contacting a LuxO polypeptide" with a σ54 polypeptide, because both of the polypeptides are inherently present in the *V. harveyi* organism and the contacting of phosphorylated LuxO with σ54-holoenzyme is inherently regulated by cell density of the culture as specifically stated in the specification (page 28). As such, the step of contacting LuxO with σ54 is inherently performed in growing cells at low density.

Freeman et al (Journal of Bacteriology, 181(3):899-906, Feb 1999) teach that cell density directly affects the light production of *V. harveyi* et al reporter strain BB120 (the same strain of the instant specification) via the expression of the lux operon (page 901, paragraph bridging columns 1-2 and Figure 2. As such, the process of growing *V. harveyi* et al at low density inherently provides for "contacting a LuxO polypeptide" with a σ54 polypeptide, because both of the polypeptides are inherently present in the *V. harveyi* organism and the contacting of phosphorylated LuxO with σ54-holoenzyme is inherently regulated by cell density of the culture as specifically stated in the specification (page 28). As such, the step of contacting LuxO with σ54 is inherently performed in growing cells at low density.

Freeman et al (Molecular Microbiology, 31(2) :665-667, January 10, 1999) teaches providing LuxO alleles containing the D47A allele to *V. harveyi* strain JAF78 to replace the Δlux-Cm^r on the chromosome, the method step of which inherently contacts the LuxO with the *V. harveyi* σ54 polypeptide.

The art is applied in view of the lack of isolation or purification of any component. Since *V. harveyi* is a free living marine organism and that the light production was measured in a test tube, the growing and detection of light in a test tube meets the apparent limitations of *in vitro* and *in vivo*, in the absence of a clear definition of *in vivo* as it relates to the "free living" marine organism *V. harveyi*. Further, the providing of the LuxO D47A allele meets the method step as it inherently contacts the LuxO allele with

the V. harveyi σ54 polypeptide. Therefore, the growth of *V. harveyi* at low cell density meets the limitation of regulating the activity of a σ54 polypeptide because the process of growing at low cell or providing the LuxO D47A allele inherently performs this step and inherently has the same claimed function as the natural process is discloses as being regulated in this manner. Further, contacting is not defined in the specification and broadly includes "brining together" in any manner. Ex parte Novitski 26 USPQ 1389 (BPAI 1993); Mehl/Biophile International Corp. V. Milgraum, 52 USPQ2d 1303 (Fed. Cir. 1999); Atlas Powder Co. V. IRECO, 51 USPQ2d 1943 (Fed. Cir. 1999).

Status of the Claims

Claims 9-12 stand rejected. Claims 1-8 and 13-53 are withdrawn from consideration.

Conclusion

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Patricia A. Duffy whose telephone number is 571-272-0855. The examiner can normally be reached on M-F 6:30 pm - 3:00 pm. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Lynette Smith can be reached on 571-272-0864.

The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

for a. Duffy
Patricia A. Duffy, Ph.D.

Primary Examiner

Art Unit 1645